

60th Medical Group (AMC), Travis AFB, CA
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
FINAL REPORT SUMMARY

(Please type all information. Use additional pages if necessary.)

PROTOCOL #: FDG20140007A

DATE: 29 July 2015

PROTOCOL TITLE: "Study of the efficacy of extracellular matrix arterial interposition grafts in a sheep (*Ovis aries*) model."

PRINCIPAL INVESTIGATOR (PI) / TRAINING COORDINATOR (TC): Maj Lucas Neff

DEPARTMENT: General Surgery



PHONE #: 423-5179

INITIAL APPROVAL DATE: 14 November 2013

LAST TRIENNIAL REVISION DATE: 20 November 2014

FUNDING SOURCE:

1. **RECORD OF ANIMAL USAGE:**

Animal Species:	Total # Approved	# Used this FY	Total # Used to Date
Ovis aries	12		

2. **PROTOCOL TYPE / CHARACTERISTICS:** (Check all applicable terms in EACH column)

<input type="checkbox"/> Training: Live Animal	<input type="checkbox"/> Medical Readiness	<input type="checkbox"/> Prolonged Restraint
<input type="checkbox"/> Training: non-Live Animal	<input type="checkbox"/> Health Promotion	<input type="checkbox"/> Multiple Survival Surgery
<input checked="" type="checkbox"/> Research: Survival (chronic)	<input type="checkbox"/> Prevention	<input type="checkbox"/> Behavioral Study
<input type="checkbox"/> Research: non-Survival (acute)	<input type="checkbox"/> Utilization Mgt.	<input type="checkbox"/> Adjuvant Use
<input type="checkbox"/> Other ()	<input checked="" type="checkbox"/> Other (Treatment)	<input type="checkbox"/> Biohazard

3. **PROTOCOL PAIN CATEGORY (USDA):** (Check applicable) ☐ C ☒ D ☐ E

4. **PROTOCOL STATUS:**

***Request Protocol Closure:**

☐ Inactive, protocol never initiated

☐ Inactive, protocol initiated but has not/will not be completed

☒ Completed, all approved procedures/animal uses have been completed

5. **Previous Amendments:**

List all amendments made to the protocol.. IF none occurred, state NONE. Do not use N/A.

For the Entire Study Chronologically

Amendment Number	Date of Approval	Summary of the Change
1	19 Dec 13	Personnel.
2	14 Jul 14	Personnel

3	6 Aug 14	Personnel
4	28 Aug 14	Procedure
5	21 May 15	Personnel
6	2 Jun 15	Other

6. **FUNDING STATUS:** Funding allocated: \$ Funds remaining: \$ 0.00

7. **PROTOCOL PERSONNEL CHANGES:**

Have there been any personnel/staffing changes (PI/CI/AI/TC/Instructor) since the last IACUC approval of protocol, or annual review? ☒ Yes ☐ No

If yes, complete the following sections (Additions/Deletions). For additions, indicate whether or not the IACUC has approved this addition.

ADDITIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, IACUC approval - Yes/No)

Maj Lucas Neff (Co-PI), Capt Hilary Loge (AI), Capt Rachel Russo (AI), Maj Timothy Williams (AI)

DELETIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, Effective date of deletion)

Lt Col Daren Danielson (PI)

8. **PROBLEMS / ADVERSE EVENTS:** Identify any problems or adverse events that have affected study progress. Itemize adverse events that have led to unanticipated animal illness, distress, injury, or death; and indicate whether or not these events were reported to the IACUC.

Three sheep died within 30 days of surgery due to wound infection, or failure of graft, or failure of anastomosis. These events were reported to the IACUC Chair.

9. **REDUCTION, REFINEMENT, OR REPLACEMENT OF ANIMAL USE:**

REPLACEMENT (ALTERNATIVES): Since the last IACUC approval, have alternatives to animal use become available that could be substituted in this protocol without adversely affecting study or training objectives?

No.

REFINEMENT: Since the last IACUC approval, have any study refinements been implemented to reduce the degree of pain or distress experienced by study animals, or have animals of lower phylogenetic status or sentience been identified as potential study/training models in this protocol?

No.

REDUCTION: Since the last IACUC approval, have any methods been identified to reduce the number of live animals used in this protocol?

No.

10. **PUBLICATIONS / PRESENTATIONS:** (List any scientific publications and/or presentations that have resulted from this protocol. Include pending/scheduled publications or presentations).

None.

11. **Were the protocol objectives met, and how will the outcome or training benefit the DoD/USAF?**

Yes. The protocol was completed despite the surgical misadventures described above, and provided a valuable GME opportunity.

12. PROTOCOL OUTCOME SUMMARY: (Please provide, in "ABSTRACT" format, a summary of the protocol objectives, materials and methods, results - include tables/figures, and conclusions/applications.)

Title: Study of the efficacy of extracellular matrix arterial interposition grafts in a sheep (*Ovis aries*) model.

Coauthors: Lucas Neff MD,¹ Sterling Humphrey MD,² Kevin Grayson DVM PhD³

¹Department of Surgery, David Grant USAF Medical Center

²Department of Surgery, UC Davis Medical Center

³Clinical Investigation Facility, David Grant USAF Medical Center

Objective: The purpose of this study was to compare the long term performance of Cormatrix™ small intestine submucosa carotid artery interposition grafts in sheep.

Methods: Twelve sheep were anesthetized, instrumented, and had 8 cm of both carotid arteries removed through a midline neck incision. Interposition grafts were created by tubularizing pressed or nonpressed CorMatrix™ extracellular matrix sheets with staples. End-to-end anastomoses were performed with polypropylene in a randomly assigned vessel and polydioxanone suture in the contralateral artery. The wounds were closed and the animals recovered. Lovenox was administered daily for 30 days. Groups of animals were survived for nine and twelve months, at which time duplex ultrasonography was conducted and the animals euthanized, followed by necropsy and histologic evaluation of the grafts.

Results: Four of twelve animals (33%) succumbed within weeks of the procedure (median 16 days, range 12 to 30 days) due to failure of an anastomosis. All four failures were sewn with polypropylene suture. Among the surviving eight sheep, six of eight grafts sewn with polydioxanone (75%) and five of eight grafts sewn with polypropylene (63%) remained patent ($p = 0.59$). The remaining grafts (two sewn with polydioxanone and three with polypropylene, respectively) were occluded by organized thrombi ($p = 0.71$). All of the patent grafts were aneurysmal with mid-graft diameters 3.1-fold (range 2.1 to 5-fold) greater than adjacent native carotid arteries. Besides being enlarged, the patent grafts were extremely stiff, so planned function testing was not possible.

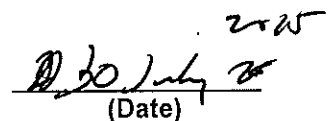
Histologically, the five occluded grafts had mature fibrotic thrombi that were undergoing recanalization. Ten of eleven (91%) patent grafts had antemortem thrombi. Four of eleven (36%) appeared to have delaminating graft material, evenly divided between pressed and unpressed products. Degenerating material was visible in five of eleven (45%) grafts, with no significant difference between pressed and unpressed products ($p = 0.82$).

Mononuclear cell infiltration was present in various amounts, forming lymphoid follicles in some grafts.

Immunohistochemical staining endothelial cells for vonWillebrand factor was only positive in two of eleven grafts (18%).

Conclusion: The tubularized stapled grafts were problematic, resulting in thrombus formation in nearly every graft. Polydioxanone anastomoses had fewer failures than polypropylene, although the difference was not statistically significant. The extracellular matrix tended to be unincorporated and delaminating or degenerating in many cases. Overall, we cannot recommend tubularized extracellular matrix interposition grafts in a sheep model.


(PI / TC Signature)


(Date)

Attachments:

Attachment 1: Defense Technical Information Center (DTIC) Abstract Submission (Mandatory)

Attachment 1**Defense Technical Information Center (DTIC) Abstract Submission**

This abstract requires a brief (no more than 200 words) factual summary of the most significant information in the following format: Objectives, Methods, Results, and Conclusion.

Objective: This study evaluated the long term performance of small intestine submucosa carotid artery grafts in sheep.

Methods: Twelve sheep had both carotid arteries removed. Grafts were created by tubularizing extracellular matrix sheets with staples and anastomosed end-to-end. Groups of animals were survived for nine and twelve months.

Results: 4 of 12 animals succumbed within weeks of the procedure due to failure of an anastomosis. Among 8 sheep, 11 of 16 grafts were patent. The remaining 5 grafts were occluded by organized thrombi. All of the patent grafts were aneurysmal with mid-graft diameters 3.1-fold greater than adjacent native carotid arteries. Ten of 11 patent grafts had antemortem thrombi. Four of 11 appeared to have delaminating graft material. Degenerating material was visible in 5 of 11 grafts. Mononuclear cell infiltration was present in various amounts, forming lymphoid follicles in some grafts. Immunohistochemical staining of endothelial cells for vonWillebrand factor was only positive in 2 of 11 grafts.

Conclusion: The tubularized stapled grafts were problematic, resulting in thrombus formation in nearly every graft. The extracellular matrix tended to be unincorporated and delaminating or degenerating in many cases. Overall, we cannot recommend tubularized extracellular matrix interposition grafts in a sheep model.

Grant Number: _____

From: _____

****If you utilized an external grant, please provide Grant # and where the grant came from. Thank you.**